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The effect of garlic oil on lipid peroxidation and blood cell counts of arsenic exposed albino mice

R. Latha[1]*, L.Venkatakrishnan[2], V. Aruna[2], G. Lavanya[2]

1. Department of Physiology
2. Department of Gastroenterology & liver unit and Biochemistry, PSG Institute of Medical Sciences and Research, Peelamedu, Coimbatore - 641 004.

Abstract

Objective: This study was undertaken to investigate the antiperoxide effect of garlic oil on various tissues and blood cell counts of arsenic exposed albino mice. Materials and methods: Garlic oil (100 mg/ kg body wt/ day) removed from garlic pearls purchased from Ranbaxy was administered orally to albino mice and its protective effect was determined on sodium arsenite-induced lipid peroxidation (LPO) of different tissues and blood cell counts of mice. Results: A significant reduction in arsenic-induced LPO in the kidney of mice was observed after the administration of garlic oil. There was a significant increase in the number of neutrophils and eosinophils in mice administered with garlic oil. A significant weight gain observed in mice given sodium arsenite (i.p.) was found to be declined after the garlic oil administration. Conclusion: These results indicate that garlic oil can be administered in the form of garlic pearls to persons who are working in places of coal burning, mining and metal ore smelting, as the diallyl disulphide (DADS) which is the active principle of garlic has a possible protective role on LPO status of certain tissues and blood cell counts of arsenic exposed mice.

Keywords: Garlic oil, Lipid peroxidation, Arsenic – exposed, Thiobarbituric acid reacting Substances, Diallyl disulphide (DADS).

1. Introduction

Arsenic ranks first in a list of 20 hazardous substances by the Agency for Toxic Substances and Disease Registry and United States Environmental Protection Agency. It is a member of the group VA element of the periodic table and 52nd most abundant element in the earth's crust. Arsenicals were used as early as 2000 BC both as medicines and poisons. Arsenic present in metal ores or coal, is released during smelting process or coal burning as flue gas or stack dust contaminating air, water and soil.

It is a potent pro-oxidant increasing the LPO in all the tissues. Recently, Chaudhuri *et al* [1] have seen that increased LPO at different doses of arsenic, decreased glutathione (GSH) level, superoxide dismutase and glutathione reductase

* Corresponding author
E-mail: lathaphysio@yahoo.co.in

activities in rats fed with drinking water mixed with arsenic. Hong *et al* [2] have found that inhalation of arsine gas (AsH_3) produces a stress on the haemopoietic system characterised by the hemolysis.

Garlic is a known anti-oxidant. Sheela *et al* [3] have found that the sulfur containing aminoacid S-allyl cysteine sulfoxide present in garlic showed significant anti-peroxide effect in high fat diet fed rats. Kritchevesky et al [4] and Rajmohan *et al* [5] put forward the hypothesis that proteins with low lysine: arginine ratio affects the atherogenecity.

In garlic protein lysine:arginine ratio is 1:1.135. This low ratio of 0.77 for these aminoacid in garlic protein could be a key factor for its reported hypolipidemic and antioxidant actions. But the antioxidant effect of garlic oil on LPO status of tissues and blood cell counts of arsenic exposed mice has not yet been investigated.

So, it was decided to study the effect of garlic oil on LPO in different tissues and blood cell counts of arsenic-exposed mice which would be useful to persons who are working in places of mines, coal burning or metal ore smelting and who are drinking arsenic contaminated water.

2. Materials and methods

2.1 Animals

All animal experiments were performed according to the ethical guidelines suggested by the Institutional Animal Ethics Committee (IAEC) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Culture, Government of India. The adult Wistar strain male albino mice 30±5g, obtained from institute animal house were used for this study. The animals were maintained on commercial feed (calcium 1%, phosphate 0.6 %) obtained from Gold Mohur Animal Feeds (Bangalore, India). Food and water were given *ad libitum.*

2.2 Drugs and reagents

Tricarboxylic acid (TCA), thiobarbituric acid (TBA) and sodium arsenite were purchased from Sigma chemical Co.(St. Louis, MO. USA). Garlic oil was removed from garlic pearls which was purchased from Ranbaxy. Each pearl contains:

Garlic oil 0.25% w/w
Excipients quantum sufficient to 250mg

2.3 Experimental procedures

The animals were randomly divided into three groups consisting of 10 mice in each group as follows,

Group I - control group and were given food and water *ad libitum.*

Group II - were administered with 2.5 mg/ kg. body wt./ day of sodium arsenite intra-peritoneally for 20 days.

Group III- were fed by oral gavage garlic oil at a dose of 100 mg/ kg body wt/ day followed by the i.p. administration of sodium arsenite (same dose) one hour later for 20 days.

All the mice were weighed both before and after the experiment to see any alteration in the weight. At the end of the experiment period, blood was collected by retro-orbital puncture by lightly anaesthetizing the animals using ether into bottles containing Ethylene Diamine Tetra Acetic acid (EDTA). And immediately the abdomen was opened by a midline incision and their lungs, heart, liver and kidneys were excised carefully, washed with chilled saline (0.9%) and stored in ice chamber [6] until analysis.

2.4 Estimation of thiobarbituric acid reacting substances (TBARS) - Ohkawa et al [7]

LPO was estimated as evidenced by the formation of TBARS. For estimation of lipid peroxide content, the tissue homogenate was prepared in 0.1 M acetate buffer pH 5.0 in Erlenmeyer flask and incubated

for 1 h (37°C). Aliquots of 1ml is taken into 1.5 ml of 20 % cold TCA centrifuged at 3000 rpm for 10 min. To 2 ml of the supernatent 2 ml of 0.67 % aqueous TBA reagent was added, mixed well and kept in a boiling water for 10 min. After cooling pink colour was measured at 535 nm. Molar extinction coefficient was 1.56×10^5/cm/M.

2.5 Determination of blood cell counts

The various blood cell counts like red blood cell (RBC), white blood cell (WBC) and differential leucocyte counts were determined using Hemocytometer consisting of Neubauer's counting chamber with appropriate diluting fluids, pipettes and the stain.

2.6 Statistical analysis

Data were expressed as mean ±SE of 8 values in each group. One- way ANOVA test was performed to find whether or not scores of different groups differ significantly. To test inter group significant difference, Student's unpaired *t* - test was performed. Statistical probability of $p<0.05$ was considered to be significant.

3. Results

3.1 TBARS

Table 1. shows that compared to control, group II mice on exposure to arsenite showed an increase in MDA production by 50% in lungs, heart and kidneys and 79% ($p<0.01$) in the liver. Administration of garlic oil, an antioxidant along with arsenite had shown to effectively reduce such arsenic-generated increase in MDA production in kidney of mice ($p<0.01$).

3.2 Blood cell counts

Even though arsenite treatment caused a moderate decrease in RBC and WBC counts, it was not statistically significant (Table 2). But garlic oil administration significantly increased the neutrophil ($p<0.01$) and eosinophil ($p<0.02$) counts under differential leucocyte count. Lymphocyte count was decreased significantly ($p<0.05$).

3.3 Body weight

As shown in table 2, there was a significant weight gain in group II ($p< 0.05$) mice, when compared with group I mice. But the garlic oil treatment decreased the weight in group III mice from that of group II mice though it was just short of reaching statistically significant level.

4. Discussion

Arsenic is a pro-oxidant [8] and may cause lipid peroxidation in many tissues. The principal natural reservoirs of arsenic are rocks. Release and mobilisation of arsenic from these sources constitute the availability of this element in soil, water and air in various forms.

As a result human are always and unavoidably exposed to this toxic metalloid. A large number

Table 1.
Effect of garlic oil on sodium-arsenite induced changes in LPO status of different tissues of mice.

Group	Treatment	Tissues (TBARS in nmol of MDA formed / gm of tissue)			
		Lungs	Liver	Heart	kidneys
I	Control	0.04 ± 0.02	0.28 ± 0.10	0.12 ± 0.01	0.12 ± 0.06
II	Arsenite-treated (2.5mg/kg body wt/day)	0.06 ± 0.03	0.50 ± 0.13***	0.18 ± 0.07	0.18 ± 0.11
III	Arsenite+Garlic oil (100mg/kg body wt/ day)	0.09 ± 0.04	0.54 ± 0.13	0.10 ± 0.08	0.04 ± 0.01***

Values are expressed as mean±S.E.; n=8.***$p<0.01$ (one-way ANOVA followed by Student's unpaired *t* - test).

Table 2.
Effect of garlic oil on sodium arsenite induced changes in blood cell counts and body weight of mice.

Gr.	Treatment	RBC (millions/ cumm)	WBC (thousands/ cumm)	Differential count (%)				Weight (% ↑ or ↓in weight)
				Neutrophil	Eosinophil	Monocyte	Lymphocyte	
I	Control	4.45± 0.41	6.60± 1.49	65.25± 7.94	1.75± 1.16	1.25± 1.38	31.75± 7.10	6.55± 1.66
II	Arsenite treated (2.5mg/kg bodywt/day)	4.10± 0.52	6.56± 0.80	61.37± 6.52	1.12± 1.12	0.62± 0.74	36.50± 6.21	13.77± 7.81*
III	Arsenite+Garlic oil (100mg/kg body wt/ day)	4.02± 0.11	6.38± 0.57	70.00± 4.30***	2.37± 0.74**	0.50± 0.53	30.50± 3.89*	8.87± 7.82

Values are expressed as mean±S.E.; n=8. ***p<0.01, **p<0.02, *p<0.05(One way ANOVA followed by Student's unpaired t- test).

of man made arsenic compounds are used in agriculture as effective agents against pests, parasites and weeds and gradually accumulated in the soil [9,10]. Arsenic present in the various metal ores or coal is released during the smelting process or in coal burning, which produces stack dust and flue gas to contaminate the soil, air and water with arsenic [11].

As a result arsenic pollution in mining places and in smelting or coal burning in thermal power plants continues to be a severe health problem and hence this study was conducted to find out whether garlic oil in the form of garlic pearls can be administered to persons who are working in places with arsenic contamination, as a prophylactic measure to protect the tissues from the lipid peroxidative effect of arsenic.

Sodium arsenite, the main toxic form of arsenic in the environment, is 60 times more toxic than sodium arsenate [12]. The significant increase in LPO following sodium arsenite administration seen in liver of mice reported in this paper agree well with the results obtained in the previous studies [13].

Ramos *et al*. [14] demonstrated that acute arsenic exposure for one hour in female rats lead to significant increase in the LPO of liver, kidney, lung and heart with concomitant decrease in tissue glutathione content. The LPO was also increased in lungs, heart and kidneys, although not at a statistically significant level.

The absence of significant LPO seen in tissues other than liver following chronic arsenic exposure for 20 days might be due to the activation of hepatic methyl transferase methylating arsenite to Monomethyl arsinic acid (MMA) and then to Dimethyl arsonic acid (DMA) by the methyl donor S-Adenosyl methionine (SAM) which is then excreted in urine [15]. Healy *et al* have reported that when mice were given arsenate in drinking water for 32 or 91 days, the arsenite methyl transferase activities of liver, testis, kidneys and lungs cytosol were increased [16].

Styblo *et al* [17] have found that the trivalent arsenicals like arsenite (As III) are preferred substrates for methylation reactions. He had also indicated that both inorganic and methylated arsenicals are bound to proteins in rat liver cytosol and in mouse liver and kidneys. The LPO seen in liver of mice could not be modulated by garlic oil administration, as Sheen *et al* [18] have detected LPO when the primary rat hepatocytes

were treated with 2mM diallyl disulphide (DADS) which is the active principle of garlic.

But it could reduce the LPO status of kidneys significantly. Borek [19] reported that aged garlic extract inhibits LPO and exerts antioxidant action by scavenging reactive oxygen species. Arivazhagan *et al* [20] have seen that administration of garlic and neem leaf extracts significantly reduced LPO and enhanced the hepatic levels of glutathione and glutathione dependant enzymes.

The oxidant effect of arsenite in the kidneys of mice is counteracted by the garlic oil by increasing the activities of antioxidant enzymes namely catalase, superoxide dismutase and glutathione peroxidase [21].

Though the RBC count is reduced after sodium arsenite administration it was not statistically significant. But the experimental studies showed significant decrease in the red cell production resulted from the administration of arsenic and arsenious acid [22]. Arsine (AsH_3) is a potent hemolytic agent and is believed to be fixed by hemoglobin in a non- volatile form within RBC after which lysis occurs [23], perhaps as the consequence of the action of compounds formed in the oxidation of arsine [24]

The insignificant change in the RBC count in group III mice confirms that the glutathione (GSH) content and the GSH- related antioxidant enzymes in RBC might be normal. This was strongly supported by Sheen *et al* [25] as the garlic components DAS (diallyl sulphide) and DADS increased GSH levels in RBC but did not influence hepatic GSH levels. This is because, the garlic oil and allyl compounds play a differential role in modulation of the GSH-related antioxidant system in tissues and red blood cells.

But the polychromatophilic neutrophils showed a significant increase following garlic oil administration compared with arsenic-treated mice. The polychromatophilic neutrophils decrease in number following sodium arsenite injection as the inorganic arsenic poisoning causes granulocytopenia [26], anaemia and less often thrombocytopenia.

The significant weight gain was seen in arsenic-treated mice. This is contradictory to the previous studies [27] where nominal dosages of sodium arsenite of 4 and 8 mg/ kg body wt./ day for 60 days caused a significant dose-dependent decrease in body weight and feed consumption, where the weight loss is caused by the decreased feed consumption and not by the direct effect of the sodium arsenite. The decrease in weight seen in group III mice from that of group II mice might be due to the hypolipidemic action of garlic oil [28].

Garlic protein contain both thiol and disulfide groups. It is also pointed out by Sanlin [29] that sulfur containing aminoacids have a special role as hypolipidemic agents. Free radical scavenging activity of garlic extract have reported by Unnikrishnan *et al* [30]. He has ascribed this property of garlic principles due to the presence of a large amount of sulf-hydryl compounds in them which scavenge OH^- radicals. The decrease in weight gain seen in group III mice was confirmed by the studies of Sheen [25], where garlic oil and DADS significantly reduced the body weight gain of rats.

5. Conclusion

It appears from the present investigation that the significant increase in LPO seen in liver after sodium arsenite injection which might be due to the protein loving nature of arsenic or due to the greater concentrating ability of arsenic by liver, could not be modulated by the administration of the garlic oil at the dose of 100mg/ kg body wt / day for 20 days. At the same time the administration of garlic oil can effectively

decrease the LPO of kidneys of mice, showing the protective effect of garlic oil on the kidneys.

Moreover, the garlic oil has an effect of causing neutrophilia and eosinophilia which would otherwise be neutropenia and eosinopenia caused by the administration of sodium arsenite. The garlic oil is able to decrease the body weight of mice, as it has organosulfur compounds such as DADS. These and related protective effects of garlic oil reported here illustrate the therapeutic effects of garlic oil.

These results indicate that the administration of garlic oil in the form of garlic pearls may be an effective prophylactic measure in persons who are working in places of coal burning, mines and metal ore smelting. Further studies may be undertaken to explore whether garlic oil of higher dose or for longer duration has the capacity to modulate the LPO status of the tissues other than kidney and blood cell counts of arsenic- exposed animals.

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